Supporting Information for:

Processive Replication of Single DNA Molecules in a Nanopore Catalyzed by phi29 DNA Polymerase

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a

b

Figure S1. DNA hairpin substrates used in bulk phase primer extension and excision assays. Sequences of the 67 mer, 14 base-pair haipin DNA substrates bearing a 5′-6-FAM label, and either an (a) 3′-OH or (b) 3′-H terminus are shown. The hairpin in (a) was used in the assay shown in Figure 2a, lanes 1-6; the hairpin in (b) was used in the assays in Figure 2a, lanes 7-12, Figure 3b, Figure 4b, and Figure 5a.

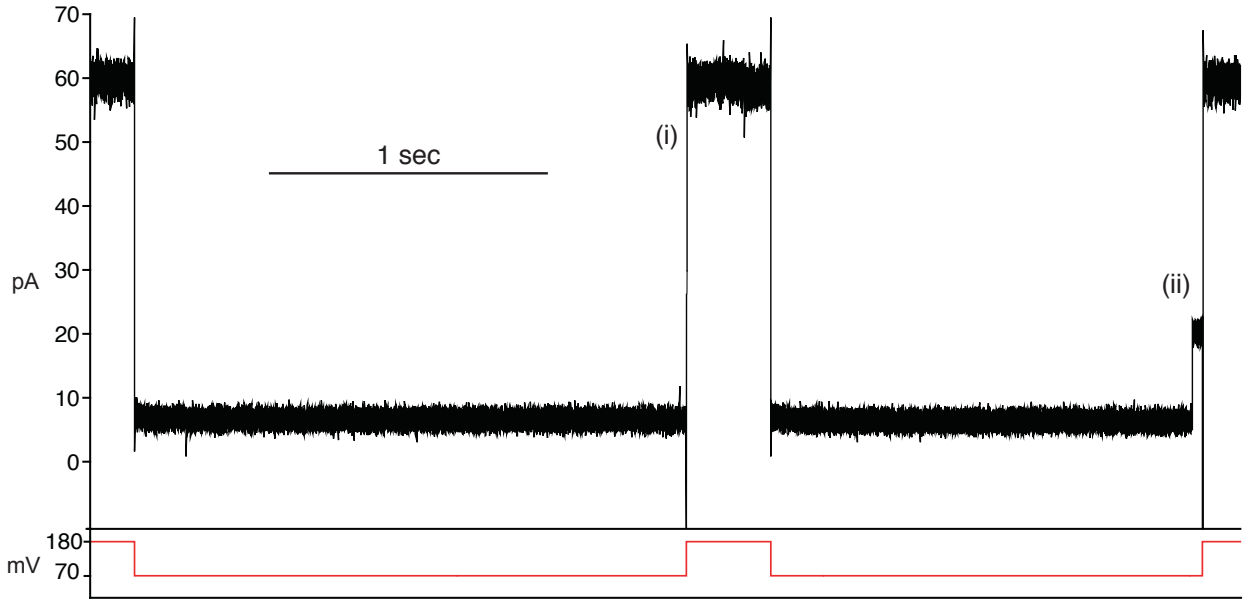

Figure S2. Unbound DNA hairpin substrates at 70 mV applied potential. Representative current traces for two 5ab(12,16) DNA hairpin molecules captured in series. The buffer was composed of 10 mM K-Hepes, pH 8.0, 300 mM KCl, 1 mM DTT and 1 mM EDTA. DNA was captured at 180 mV applied potential. If the current dropped below 45 pA for at least 0.5 ms, a finite state machine (FSM) reduced the applied potential to 70 mV for a maximum of two seconds. The FSM restored the applied potential to 180 mV after either (i) two seconds had passed at 70 mV, or (ii) the current increased to 20 +/- 2 pA (the open channel current at 70 mV) for at least 0.5 ms, indicating that the DNA had electrophoresed through the channel to the *trans* chamber.

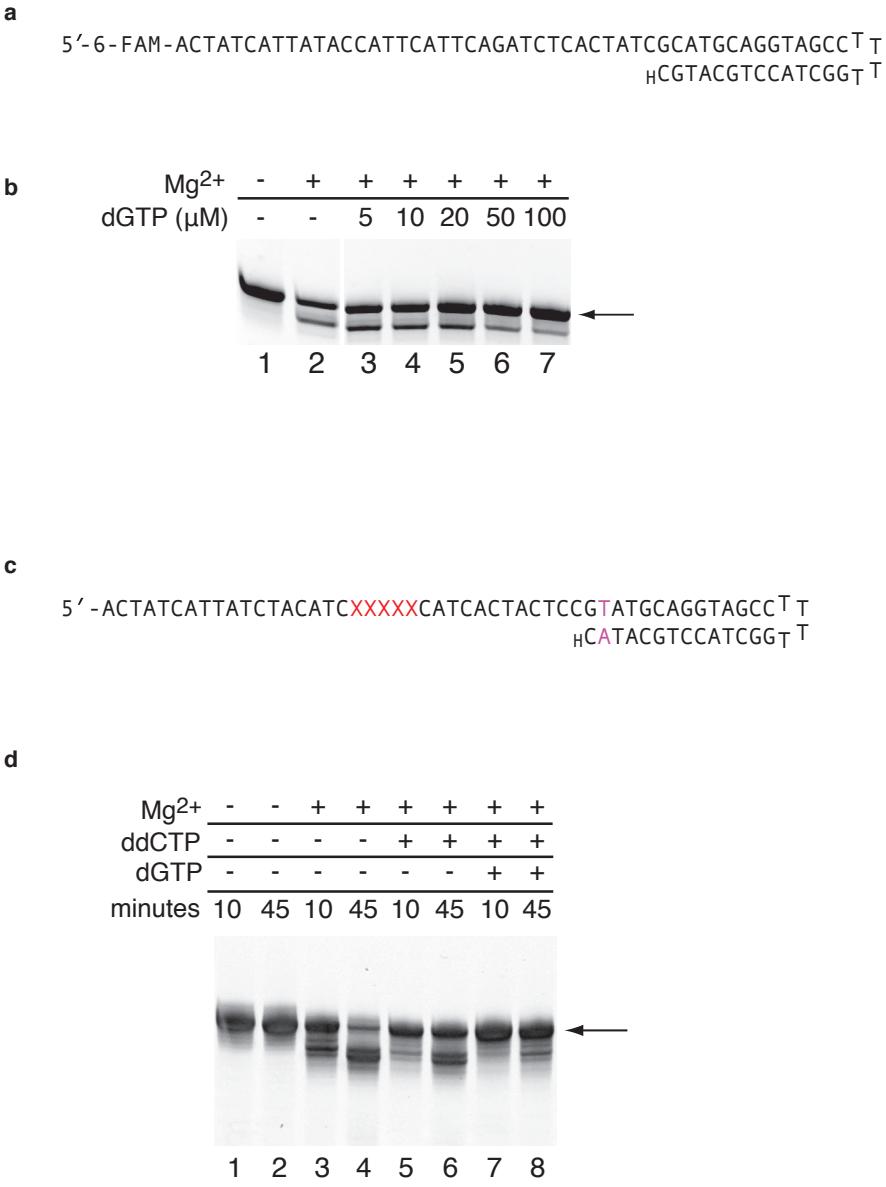

Figure S3. Protection of 3′-H-terminated DNA hairpin substrates from phi29 DNAP-catalyzed exonucleolytic digestion in the presence of complementary incoming dNTP (DNAP-DNA-dNTP ternary complex formation). (a) 5′-6-FAM labeled, 14 base-pair DNA substrate bearing a 3′-H terminus. (b) The DNA substrate shown in panel a was incubated for 45 minutes at room temperature with 0.75 μM phi29 DNAP in buffer containing 1 mM EDTA, absent (lane 1) or present (lanes 2-7) 10 mM $MgCl_2$, with dGTP (complementary to the n=0 template position in the polymerase active site) present at the indicated concentrations. In the absence of ddCTP to restore the primer terminus upon excision, the most plausible mechanism for the protection

afforded by dGTP in this assay is ternary complex formation that increases the proportion of time the primer terminus spends in the polymerase domain rather than in the 3′-5′-exonuclease domain. (b) 14 base-pair 5ab(12,16) DNA hairpin bearing a 3'-H terminus, in which the -2 base-pair was changed from the G-C pair (primer strand-template strand) present in the other DNA substrates used in this study, to an A-T pair. (d) The DNA substrate shown in panel c was incubated at room temperature with 0.75 μM phi29 DNAP in buffer containing 1 mM EDTA, absent (lanes 1 and 2) or present (lanes 3-8) 10 mM $MgCl_2$ for 10 or 45 minutes as indicated. ddCTP (400 μM) or dGTP (100 μM) were present as indicated. In contrast to substrates with a G-C pair at -2, with this substrate if the -1 (ddCMP) and -2 (dAMP) nucleotides are both excised by phi29 DNAP, the full-length hairpin cannot be regenerated in the presence of ddCTP. Thus the most plausible mechanism for the protection afforded by dGTP in this assay is ternary complex formation. Reaction products in panels b and d were resolved on 18% denaturing polyacrylamide gels. The gel in panel d was stained with Sybr Gold (Invitrogen). Gels were visualized on a UVP Gel Documentation system using a Sybr Gold filter (485-655 nm). In panels b and d, the arrows indicate the gel bands corresponding to the intact full-length DNA substrate.

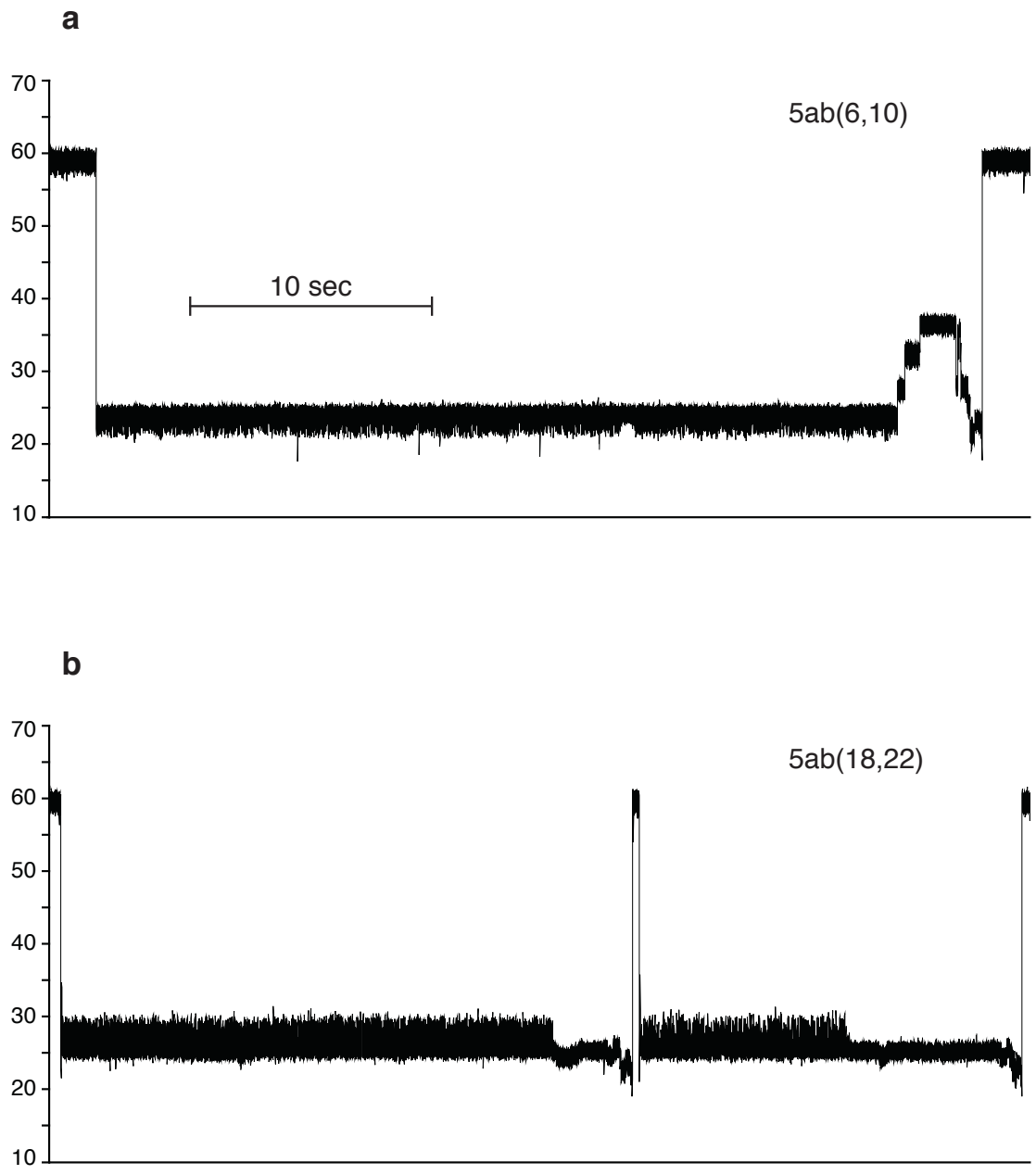

Figure S4. Amplitude steps in the terminal cascade vary as a function of initial DNA substrate abasic configuration. Representative capture events at 180 mV for phi29 DNAP-DNA binary complexes formed with DNA hairpin substrates with abasic configurations (a) 5ab(6,10) or (b) 5ab(18,22). Complexes were formed in buffer containing 10 mM K-Hepes, pH 8.0, 300 mM KCl, 1 mM DTT and 1 mM EDTA, with no added $MgCl_2$.

Complete author lists for references 4 and 5:

(4) Eid, J.; Fehr, A.; Gray, J.; Luong, K.; Lyle, J.; Otto, G.; Peluso, P.; Rank, D.; Baybayan, P.; Bettman, B.; Bibillo, A.; Bjornson, K.; Chaudhuri, B.; Christians, F.; Cicero, R.; Clark, S.; Dalal, R.; Dewinter, A.; Dixon, J.; Foquet, M.; Gaertner, A.; Hardenbol, P.; Heiner, C.; Hester, K.; Holden, D.; Kearns, G.; Kong, X.; Kuse, R.; Lacroix, Y.; Lin, S.; Lundquist, P.; Ma, C.; Marks, P.; Maxham, M.; Murphy, D.; Park, I.; Pham, T.; Phillips, M.; Roy, J.; Sebra, R.; Shen, G.; Sorenson, J.; Tomaney, A.; Travers, K.; Trulson, M.; Vieceli, J.; Wegener, J.; Wu, D.; Yang, A.; Zaccarin, D.; Zhao, P.; Zhong, F.; Korlach, J.; Turner, S. Real-Time DNA Sequencing From Single Polymerase Molecules. *Science*. **2009,** *323*, 133-38.

(5) Harris, T. D.; Buzby, P. R.; Babcock, H.; Beer, E.; Bowers, J.; Braslavsky, I.; Causey, M.; Colonell, J.; Dimeo, J.; Efcavitch, J. W.; Giladi, E.; Gill, J.; Healy, J.; Jarosz, M.; Lapen, D.; Moulton, K.; Quake, S. R.; Steinmann, K.; Thayer, E.; Tyurina, A.; Ward, R.; Weiss, H.; Xie, Z. Single-Molecule DNA Sequencing of a Viral Genome. *Science*. **2008,** *320*, 106-09.